

STATISTICAL MEASURES OF INTERSPECIFIC ASSOCIATION BETWEEN THE FLEAS OF THE GRAY-TAILED VOLE, *MICROTUS CANICAUDUS* MILLER¹

G. David Faulkenberry², Richard G. Robbins³

ABSTRACT: Statistical methods for analyzing interspecific associations of vertebrate ectoparasites are discussed. The o- and Q-statistics, based on relative odds, are used to measure the degree of association between different flea species on the gray-tailed vole, *Microtus canicaudus* Miller. These statistics are shown to be preferable to the negative correlation factor.

The gray-tailed vole, *Microtus canicaudus* Miller, sometimes considered a subspecies of the montane vole *Microtus montanus* (Peale), occurs abundantly in grassy, uncultivated fields between the Cascade and Coast Ranges of western Oregon and Washington (Hall and Kelson, 1959; Ingles, 1965; Maser and Storm, 1970). From February 1973 to January 1974, a study was made of the population dynamics and ecology of the fleas that parasitize this vole in the vicinity of Corvallis, Oregon (Robbins, 1976). Five hundred and eleven fleas representing eight species and three families were recovered from the pelts of 377 comparably collected voles. *Catallagia charlottensis* (Baker) was by far the most abundant flea present (252 specimens) and together with *Athyphloceras multidentatus* (C. Fox) (100 specimens) accounted for nearly 70% of the specimens collected. Other species, in order of abundance, were *Peromyscopsylla selenis* (Rothschild) (62 specimens), *Monopsyllus wagneri* (Baker) (44 specimens), *Hystrichopsylla occidentalis* Holland (25 specimens), *Nosopsyllus fasciatus* (Bosc d'Antic) (14 specimens), *Corrodopsylla curvata* (Rothschild) (11 specimens), and an undetermined *Rhadinopsylla* (3 specimens).

Ordinarily, the gray-tailed vole constructs its nest in a chamber located 15-30 centimeters below the surface of the ground (Pearson, 1972); however, should objects be present at the surface the vole will build under these. For this study, wide wooden panels were scattered at random over three ecologically similar collecting sites, and at regular monthly intervals

¹Received: June 20, 1979.

²Department of Statistics, Oregon State University, Corvallis, OR 97331

³Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560

voles that had been observed building nests under these panels were captured by hand. Each vole was then immediately transferred to a large, labeled plastic jar containing fresh grass clippings and pieces of fruit. All jars were fitted with wire mesh lids. Because some voles died or injured themselves in transit or were found naturally injured in the field, only 377 of 428 voles collected in this manner could be used in the statistical tests that follow.

In the laboratory, all voles were killed by quickly wrapping them in cotton blankets saturated with chloroform. This technique prevented the escape of any ectoparasites and preserved them in the positions they had occupied on their host's body while it was alive. All fleas were recovered by vigorous brushing and careful searching of the pelt, a process that generally required half an hour per animal. To prevent flea loss, these operations were performed against a light-colored background.

Holland's (1949) argument that humidity and temperature are the principal factors influencing flea populations was confirmed by Parker (1958) in a survey of fleas on the antelope ground squirrel, *Citellus leucurus leucurus* (Merriam). This argument has also been repeatedly confirmed in the laboratory. Working with several species of unfed adult fleas, Leeson (1932) demonstrated that high temperatures and low humidities tend to shorten life while, conversely, low temperatures and high humidities prolong life. This is especially true of the lightly sclerotized preimaginal stages of fleas which are extremely sensitive to the saturation deficiency or drying power of the air. Petrie and Todd (1923), Uvarov (1931), Mellanby (1933), Edney (1947), Sharif (1948), and Humphries (1967) all observed that at high saturation deficiencies there is a pronounced increase in the death rate among larvae and pupae.

Although the present study is based on only one year of field work, flea populations at all three collecting sites experienced similar seasonal fluctuations that were positively correlated with humidity and negatively correlated with temperature (surface meteorological data compiled for Corvallis by the National Climatic Center, U.S. Department of Commerce). The Willamette Valley in which Corvallis is situated is an area of warm, dry summers and cool, wet winters. For this reason, infested voles were most often collected during the mild spring and early winter months, while uninfested voles predominated during summer and midwinter. Only 198 (52.6%) of the voles taken over the 12-month collecting period were infested, and of these 119 (60.1%) carried one flea species, 59 (29.8%) carried two, and 20 (10.1%) carried three or more. Such low infestation rates suggest that competition on the host animal is not a factor in determining species abundance; rather, the complex web of selective pressures and biotic relationships characteristic of the nest probably constitutes the regulatory mechanism. Regardless of cause, it is of interest

to know the extent to which different flea species may be expected to occur together on their host, and this paper presents statistical methods for analyzing such interspecific associations.

Data Summary and Analysis

Six of the eight flea species collected during this study were uncommon or accidental on the gray-tailed vole. It was therefore convenient to treat these six species as one with the result that only three species categories were considered. The first of these, category A, contained all specimens of *Atyphloceras multidentatus*; category C contained all *Catallagia charlottensis*; and category O contained all other flea species. The eight possible combinations of these categories are listed in Table 1 together with the observed and expected number of voles per combination. A bar over a letter indicates the absence of that category. Thus, 13 voles were infested with A, C and O, while 30 voles were infested with C and O but not with A.

Table 1. Numbers of gray-tailed voles infested with different combinations of flea species.

Flea Species Combination	Observed Number of Voles	Expected Number of Voles*
ACO	13	5.55
$\bar{A}CO$	30	25.20
$A\bar{C}O$	10	11.05
$AC\bar{O}$	22	17.18
$\bar{A}\bar{C}O$	39	50.20
$A\bar{C}\bar{O}$	23	34.23
$\bar{A}C\bar{O}$	61	78.07
$\bar{A}\bar{C}\bar{O}$	179	155.52
Totals	377	377.00

*Expected numbers are calculated from the hypothesis of independence.

The data in Table 1 imply that A was present on 18.0% of the voles collected for this study, C was present on 33.4%, and O was present on 24.4%. Expected frequencies were calculated from the hypothesis that each flea species behaves independently; that is, the probability of obtaining a vole infested with species A is unaffected by the presence or absence of C and O. To illustrate the calculations for this hypothesis, the expected number of voles with the combination $\bar{A}C\bar{O}$ is

$$nP(\bar{A})P(C)P(O) = (377) \left(\frac{309}{377}\right) \left(\frac{126}{377}\right) \left(\frac{92}{377}\right) = 25.20$$

If events \bar{A} , C and O occur independently, then the probability of their joint occurrence is the product of their marginal probabilities.

It is clear from Table 1 that all three species categories occurred together with greater frequency than would be expected under the hypothesis of independence. In addition, pairs of categories occurred more often than expected (with the exception of $A\bar{C}O$), and the number of uninfested voles was greater than expected. On the other hand, voles infested with only one species category were collected less often than expected in every case. These observations seem to indicate positive association between species. To further investigate this possibility, it is necessary to employ two-way tables such as those shown in Table 2.

Table 2. Two-way associations.

A x C			A x O			C x O		
	C	\bar{C}		O	\bar{O}		O	\bar{O}
A	35	33	68	A 23	45	68	C 43	83
\bar{A}	91	218	309	\bar{A} 69	240	309	\bar{C} 49	202
	126	251	377		92	285	377	92
								285
								377
Q = .435			Q = .280			Q = .362		
o = 2.54			o = 1.78			o = 2.14		
$X_c^2 = 11.176$			$X_c^2 = 3.392$			$X_c^2 = 8.924$		

The Q- and o-statistics appearing under each two-way table are widely used measures of association based on relative odds. The Q-statistic is obtained as follows:

$$Q_{AC} = \frac{\frac{35}{91} - \frac{33}{218}}{\frac{35}{91} + \frac{33}{218}} = \frac{(35)(218) - (33)(91)}{(35)(218) + (33)(91)} = .435$$

This statistic compares the relative odds of obtaining a vole that is infested with A when C is present and when C is absent. If the odds of obtaining a vole that is infested with A are higher when C is present, then Q will be positive, meaning that there is a positive association in the table. The range for Q is -1 to $+1$ (the same as the product-moment correlation coefficient for continuous data) where -1 indicates that the two flea categories never occur together or they are never both absent and $+1$ indicates that one category is observed only in the presence of the other. A Q-value of zero indicates no association in the sense that the odds of obtaining one category are the same whether or not the other is present. In Table 2, all Q-values are positive.

To obtain the o-statistic, it is necessary to calculate the ratio of the odds. For example, in the A x C table, the odds of obtaining A with C present are 35/91, while the odds of obtaining A with C absent are 33/218. The ratio of these odds is

$$o_{AC} = \frac{35/91}{33/218} = \frac{(35)(218)}{(33)(91)} = 2.54$$

Here, the odds of obtaining A when C is present are 2.54 times greater than when C is absent — a clear indication of positive association. An o-value of 1 indicates independence (*i.e.*, the odds of obtaining A are the same for C and \bar{C}), and an o-value less than 1 corresponds to the concept of negative association. Fleiss (1973) presents further discussion of the Q- and o-statistics and provides methods for calculating confidence intervals; Goodman and Kruskal (1954) discuss the Q-statistic as well as other measures of association.

Either o or Q may be used as a measure of association, but to formally test for independence the chi-square test (X^2) is used. The calculated chi-square values, using the continuity correlation factor (Fleiss, pp. 19-20), are given in Table 2 for each two-way table. It is clear that each pair of categories in Table 2 is positively associated. A x C and C x O show the highest degrees of association with X^2 tests significant at the .005 level, and A x O is only somewhat less positive with a X^2 significant at .10.

Such two-way tables, where two categories are summed over the third category, serve to illustrate common measures of association. However, the usual order of statistical analysis involves first testing for second order interaction to determine whether it is reasonable to sum over the third category. Testing for second order interaction means testing the hypothesis that the association of two categories is the same in the presence or absence of the third category. If the hypothesis is not rejected, then it is reasonable to form two-way tables by summing over the third category. Thus, for the categories A x C with O present and absent, the o-values are 1.69 and 2.81, respectively. While these values differ somewhat, they both indicate association in the same direction, that is, they are both greater than 1, and if they are compared using Plackett's (1962) test for second order interaction, the resulting $X^2 = .742$ with 1 degree of freedom. Since this value is not significant, there is no evidence of second order interaction.

Fluctuations in Flea Populations

As mentioned earlier, high temperatures and low humidities corresponded with a low percentage of infested gray-tailed voles during summer months, thereby increasing the frequency of the combination

$\bar{A} \bar{C} \bar{O}$. Unless taken into account, fluctuations in ectoparasite populations, whatever their cause, can lead to spuriously high but nonetheless statistically significant positive measures of association. To obtain a true measure of interspecific association, all sample data must be comparable. To approximate this condition, the data for the months May through September during which only 87 infested voles were observed have been excluded from Table 3, which is otherwise similar to Table 1. In Table 3, combinations $A C O$, $\bar{A} C O$ and $A C \bar{O}$ again occur more often than expected, while $A \bar{C} O$ occurs less often than expected. The number of uninfested voles remains greater than expected, and the number of voles bearing only one flea category is less than expected in each case.

Table 3. Numbers of gray-tailed voles infested with different combinations of flea species — excluding months May through September.

Flea Species Combination	Observed Number of Voles	Expected Number of Voles
ACO	13	7.42
$\bar{A}CO$	29	25.70
$A\bar{C}O$	9	11.63
$AC\bar{O}$	21	17.90
$\bar{A}\bar{C}O$	34	40.25
$A\bar{C}\bar{O}$	22	28.04
$\bar{A}\bar{C}\bar{O}$	50	61.78
$\bar{A}C\bar{O}$	112	97.08
Totals	290	289.80

Again, the Plackett test for second order interaction is not significant, meaning that two-way tables for each pair of categories can be formed by summing over the third category. These two-way tables appear in Table 4. In each case, the odds ratio is greater than 1 (though less than the corresponding measure in Table 2), indicating that the number of voles infested with one flea category is higher when another flea category is also present. The chi-square statistic is significant for $A \times C$ and $C \times O$ but is not significant for $A \times O$.

Table 4. Two-way associations — excluding May through September.

$A \times C$			$A \times O$			$C \times O$		
	C	\bar{C}		O	\bar{O}		O	\bar{O}
A	34	31	A	22	43	C	42	71
\bar{A}	79	146	\bar{A}	63	162	\bar{C}	43	134
	113	177		85	205		85	205

$$\begin{aligned} o &= 2.03 \\ X_c^2 &= 5.569 \end{aligned}$$

$$\begin{aligned} o &= 1.32 \\ X_c^2 &= .574 \end{aligned}$$

$$\begin{aligned} o &= 1.84 \\ X_c^2 &= 4.913 \end{aligned}$$

Whether positive or negative, the interspecific relations between parasitic arthropods may be interpreted in many ways. In the case of the gray-tailed vole, there is evidence that temperature and humidity are the chief factors regulating flea populations. Because the several flea species associated with this vole are abundant only during months of mild, wet weather, they tend to be found together or not at all. Measures of interspecific association are liable to change with time, and therefore attempts to provide such measures must not be limited to one season. However, exaggerated measures may result if data are collected without regard to population fluctuations.

Allred's Negative Correlation Factor

Allred (1971) presented an analysis of mammalian ectoparasite associations in which he used a measure that he called the "negative correlation factor," defined as follows:

Negative Correlation Factor =

$$\frac{(\text{Expected Infestation Rate}) - (\text{Actual Infestation Rate})}{\text{Expected Infestation Rate}}$$

"where the expected rate equals the sum of the actual rates of infestation of the respective, individual groups". Any two ectoparasite species A and B may occur in the following proportions:

		Species B	
		Present	Absent
Species A	Present	P_{11}	P_{12}
	Absent	P_{21}	P_{22}

Therefore, the negative correlation factor may be redefined as

$$\frac{(P_{12} + P_{21}) - P_{11}}{P_{12} + P_{21}}$$

Allred multiplied this factor by 100 to generate a range extending from $-\infty$ to 100. Large values were said to signify a lack of association; however, no distributional properties were given or referenced. In addition, this statistic has no intuitive feature, as does the odds ratio, making it difficult to determine what a particular value of the negative correlation factor means.

A further difficulty posed by use of the negative correlation factor is illustrated in Table 5 where identical negative correlation factors result from radically different data sets. Here, the negative correlation factor dictates that each data set receive the same interpretation. However, in data

Table 5. Identical negative correlation factors obtained from different data sets.

Data Set X	A	B	\bar{B}	200	Data Set Y	A	B	\bar{B}	40
	A	40	160			A	40	0	
	\bar{A}	160	640			\bar{A}	320	640	
		200	800	1000			360	640	1000
N.C.F. = 87.5					N.C.F. = 87.5				
$o = 1$					$o = \infty$				
$Q = 0$					$Q = 1$				

set X, 20% of the host animals are infested with species A regardless of the presence or absence of species B. In other words, each ectoparasite species is behaving independently, a fact that may be verified by use of the o - and Q -statistics. On the other hand, in data set Y, species A infests 4% of the host animals and species B infests 36%. Species B is far more abundant with the result that B is observed on animals that are not infested with A but A is never observed on animals that are not also infested with B. This complete association of A with B is indicated by the extreme values of o and Q . Because the negative correlation factor fails to discriminate between such data sets, it is inadequate as a measure of association and should be discarded in favor of standard statistical measures such as the o - and Q -statistics.

ACKNOWLEDGEMENTS

The junior author sincerely thanks Professor Gerald W. Krantz, Department of Entomology, Oregon State University, Corvallis, for his enthusiastic support of this research. Special appreciation is also extended to Mr. Richard F. Hoyer who collected the voles used in this study. Dr. Vernon J. Tipton of the Center for Health and Environmental Studies, Brigham Young University, kindly determined all of the flea species discussed herein.

LITERATURE CITED

- Allred, C.M. 1971. Mammalian ectoparasite consortism at the National Reactor Testing Station. *Great Basin Nat.* 31:77-82.
- Edney, E.B. 1947. Laboratory studies on the bionomics of the rat fleas, *Xenopsylla brasiliensis* Baker and *X. cheopis* Roths. II. Water relations during the cocoon period. *Bull. Ent. Res.* 38:263-280.
- Fleiss, J.L. 1973. Statistical methods for rates and proportions. John Wiley & Sons, New York.
- Goodman, L.A., and W.H. Kruskal. 1954. Measures of association for cross classifications. *J. Am. Stat. Assoc.* 49:732-764.
- Hall, E.R., and K.R. Kelson. 1959. The mammals of North America. Vol. 2. Ronald Press, New York.
- Holland, G.P. 1949. The Siphonaptera of Canada. Canada Dept. Ag. Tech. Bull. No. 70. 306 pp.
- Humphries, D.A. 1967. The behaviour of fleas (Siphonaptera) within the cocoon. *Proc. Royal Ent. Soc. London (Ser. A)* 42:62-70.

- Ingles, L.G. 1965. Mammals of the Pacific States. Stanford Univ. Press, Stanford, California.
- Leeson, H.S. 1932. The effect of temperature and humidity upon the survival of certain unfed rat fleas. *Parasit.* 24:196-209.
- Maser, C., and R.M. Storm. 1970. A key to the Microtinae of the Pacific Northwest (Oregon, Washington, Idaho). Oregon State Univ. Book Stores, Inc., Corvallis.
- Mellanby, K. 1933. The influence of temperature and humidity on the pupation of *Xenopsylla cheopis*. *Bull. Ent. Res.* 24:197-202.
- Parker, D.D. 1958. Seasonal occurrence of fleas on antelope ground squirrels in the Great Salt Lake Desert. *J. Econ. Ent.* 51:32-36.
- Pearson, J.P. 1972. The influence of behavior and water requirements on the distribution and habitat selection of the gray-tailed vole (*Microtus canicaudus*) with notes on *Microtus townsendii*. Ph.D. thesis, Oregon State Univ., Corvallis. 56 numbered pp.
- Petrie, G.F., and R.E. Todd. 1923. Reports and notes of the Public Health Laboratories, Cairo. Plague Rpt., Cairo, Egypt. 114 pp.
- Plackett, R.L. 1962. A note on interactions in contingency tables. *J. Royal Stat. Soc., Ser. B* 24:162-166.
- Robbins, R.G. 1976. A quantitative survey of the fleas associated with the gray-tailed vole, *Microtus canicaudus* Miller. Master's thesis, Oregon State Univ., Corvallis. 102 numbered pp.
- Sharif, M. 1948. Effects of constant temperature and humidity on the development of the larvae and pupae of the three Indian species of *Xenopsylla* (Insecta, Siphonaptera). *Phil. Trans. Royal Soc. London (Ser. B)* 233:581-633.
- Uvarov, B.P. 1931. Insects and climate. *Trans. Royal Ent. Soc. London* 79:1-247.

BOOK REVIEW

HOW TO KNOW THE BEETLES. 2nd ed. R.H. Arnett, Jr., N.M. Downie & H.E. Jaques. 1980. Wm. C. Brown Co., Dubuque, Iowa. 416 pp. \$9.70.

This revision of Jaques' original (1951) edition of the same title has been expanded to include representatives of *all* North American families of Coleoptera. This very desirable addition makes this edition more complete and comprehensive than its predecessor. Another desirable addition is the inclusion of a brief listing of some general references on beetles.

Throughout the text, the authors have done a good job of updating nomenclature changes which have occurred over the past 30 years. However, it is unfortunate they apparently did not have families reviewed by family authorities. This could have prevented possible errors such as those listed under *C. scutellaris* (pg. 68), an exact word carry-over from the 1st edition. A review would have dropped *modesta*, merely a melanic phase of also mentioned *rugifrons*, and also would have dropped *nigrior*, a melanic form of previously listed *unicolor*. This then might have allowed other subspecies as *lecontei* Hald. to be included in their stead.

In the main, illustrations in this edition are simple enlargements of those in the earlier edition, but the enlargements apparently were made to fit a pre-determined set space rather than any consideration being given to scale or relation to actual size of the specimen. One example of the resulting misconception of size relationships is seen when one compares the illustration of *Ataenius spretulus* (Hald.) (Fig. 423) whose 54 mm. illustration depicts a 4-5 mm. insect while a 45-60 mm. ♂ specimen of *Dynastes tityus* (L.) (Fig. 438) is depicted in a 38 mm.

(continued on page 109)